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Blood protein variation in blackbuck (*Antilope cervicapra*), a lekking gazelle

By A. Schreiber, P. Fakler, and R. Østerballe

Zoologisches Institut der Universität Heidelberg, Heidelberg, Germany and Løveparken Zoo Givskud, Give, Denmark

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Abstract

The electrophoretic screening of blood proteins representing products of 40 loci in 49 zoo-living blackbuck individuals (*Antilope cervicapra*) revealed polymorphism of transferrin, post-transferrin-2, and glucose phosphate isomerase-1, resulting in a percentage polymorphism of P = 0.075 (P = 0.034 for enzyme loci only), and an observed heterozygosity of H = 0.025 (H = 0.005 for enzymes only). These values suggest low protein polymorphism for *Antilope* when compared with most *Gazella* species. Inbreeding in captivity may explain this observation, or genetic drift in a lekking bovid with strong polygynous promiscuity. Apparently being a phylogenetic offshoot whose phenotype deviates markedly from an ancestral *Gazella*-like state chiefly in sexually selected characters, the tachytelic blackbuck lineage could have lost alleles since diverging from *Gazella*.

Introduction

The Indian blackbuck or sasin (*Antilope cervicapra*) is a gazelle (Antilopinae, Bovidae) inhabiting arid semideserts to mesic grasslands in India, Pakistan, and Nepal. Other than the controversial taxonomy of the approximately 16 species (and over 50 described taxa) in the central genus *Gazella* (e. g. Rostron 1972; Roberts 1977; Groves 1985, 1988; Furley et al. 1988; Vassart 1994), the systematic classification of blackbuck in a monotypic genus has not been contested until recently (Vassart 1994; Vassart et al. 1995 b). *Antilope* differs from *Gazella* by males having anticlockwise spiralled and rather inclined horns and conspicuous black coats sharply contrasting with their countershading white underparts; does are tawny or reddish-yellow (Haltenorth 1963; Roberts 1977). Compared with other bovid species, the relative length of horns of male blackbuck can reach remarkable dimensions when referenced to body height (Ranjitsinh 1989). Mainly to account for this sexual dimorphism, some taxonomists accepted tribus rank (Antilopini) to separate blackbuck from other gazelles. A characteristic translocation of the X-chromosome with one autosome, and many exclusive Robertsonian fusions are found in both blackbuck and *Gazella* as possible synapomorphies (Sharma et al. 1974; Effron et al. 1976; Gallagher and Womack 1992; Vassart et al. 1995 b). Osteology (Groves 1985) and allozyme data (Vassart 1994) were reported as a confirmation that blackbuck has descended from true gazelles (genus *Gazella*). The close relationship of sasin and gazelles is confirmed by successful hybridization even in free-ranging stocks: In the Gajner area (Bikaner, India), released male blackbuck were observed to court chinkara (*Gazella bennetti*) females; two viable female hybrids raised from a chinkara father and a sasin mother were sterile (Ranjitsinh 1989).

Blackbuck's close affinities with *Gazella* can hardly be doubted considering the available database, but the cladistics of their relationship remains unresolved. Effron et al. (1976) observed five putatively apomorphic chromosomal homologies uniting dama and Grant's gazelles (*Gazella dama* and *G. granti* of the subgenus *Nanger*) with *Antilope* to the reported exclusion of other *Gazella*-species. Vassart (1994) and Vassart et al. (1995 b) inferred from allozymes and chromosomal banding patterns either the clades comprising mountain (*G. gazella*) and dorcas gazelles (*G. dorcas*) or goitred (*G. subgutturosa*) and Cuvier's gazelles (*G. cuvieri*) as the most probable sister groups, and offered very robust bootstrap values in support of this inference. Morphological evidence confirms sasin's origin from a group of medium-sized gazelles, e. g. *G. dorcas*, *G. gazella*, Indian (*G. bennetti*) or Speke's (*G. speki*) gazelles (Vassart 1994). If one of these phylogenetic models is true, some species of *Gazella* should be more distantly related among each other than they are with *Antilope*, and blackbuck should have experienced accelerated evolution of its phenotype.

Blackbuck is the only gazelle in which arena display in lekking grounds has been observed, a system of mate choice where few dominant males monopolize reproduction (Ranjitsinh 1982, 1989). The resulting reproductive dominance of a limited number of males may diminish the genetic variation in populations and accelerate the fixation of phenotypic traits by sexual selection or genetic drift (Fisher 1930; Lande 1981). Despite the availability of models to quantitate the effect of male reproductive variance on genetic variation, few investigations attempted to correlate empirical molecular variability with mammalian mating systems (Apollonio and Hartl 1993; Sugg et al. 1996). The provision of blood samples from zoo-living blackbuck prompted us to compare protein variability in this polygynous ruminant with published data sets from several true gazelles to assess whether a gazelline with an untypical social system displays unusual population genetic patterns too.

Material and methods

Animals

This study is based on 49 blackbuck, i. e. 29 blackbuck kept at Givskud Zoo (Denmark), eleven from Cologne Zoo (Germany), five from Ålborg Zoo (Denmark), three from Rostock Zoo (Germany), and one from the Ménagerie du Jardin des Plantes, Paris (France). The herd at Givskud Zoo (previously kept at Copenhagen Zoo) descends from several imports to Copenhagen Zoo before 1950. Inbred over many years, an unrelated male was introduced in 1973 from Rotterdam Zoo, and a related male in 1974 from Ålborg Zoo. In 1993, the Givskud Zoo group was expanded by an unrelated breeding buck from Magdeburg Zoo. Sasin at Ålborg Zoo also descend from the Copenhagen lineage in the 1960s, but imports from Zürich Zoo (1983) and Artis Amsterdam (1993) were included. The Cologne group had been founded by animals obtained from Rotterdam and Munich Zoos, the sampled specimens being either imported individuals or their first-generation crosses. Blackbuck at Rostock Zoo descend from two male and one female founders from Berlin Zoo (1968), two males and seven females imported from India (1968), one pair from Djurpark Kolmarden, one male from Tierpark Hoyerswerda (1976), and two males and one female from Copenhagen Zoo (1979).

Blood sampling and electrophoresis

Blood samples (EDTA or acid citrate dextrose) were collected from the jugular vein, express-mailed to the laboratoy, and centrifugated immediately upon reception. Haemolysates and plasma were stored at –70 °C. Sonicated haemolysates were assayed in horizontal agarose gels, using the buffer conditions listed in table 1. Plasma proteins were resolved by PAGE in 12% polycrylamide resolution gels with 0.1% % N,N′-methylene-bis-acrylamide, 95 mM Tris and 13 mM citric acid (stacking gel: 4% acrylamide, 0.3% N,N′-methylene-bis-acrylamide, 190 mM Tris, 25 mM citric acid), using a discontinuous buffer sys-

tem (tray buffer: 66 mM Tris/32 mM boric acid pH 8.6), and visualized by staining in 0.06% Coomassie blue (in 40% methanol, 10% acetic acid, 50% aqua dest.). Prior to Coomassie-staining, PAGE-gels were assayed for plasma glutamate dehydrogenase. Zymogramme staining recipes followed Harris and Hopkinson (1976).

Results

Allozymes and structural blood proteins representing the products of 40 genetic loci were resolved in up to 49 blackbuck from five zoological gardens. Thirty-seven loci were monomorphic, including haemoglobins, albumin, postalbumin-1, posttransferrin-1 and -3, plasma glutamate dehydrogenase, and nineteen erythrocyte allozymes representing 28 loci: Acid phosphatases (Acp-1*, Acp-2*), adenosine deaminase (Ada*), adenylate kinase (Ak-1*, Ak-2*), carbonic anhydrase (Ca*), NADH-diaphorase (Dia-1*, Dia-2*, Dia-3*), erythrocyte esterases (Es-1*, Es-2*, Es-3*, substrate: methyl-umbelliferyl acetate), glucose-6-phosphate dehydrogenase (G6pdh*), glucose phosphate isomerase (Gpi-2*), glutamate oxaloacetate transaminase (Got*), isocitrate dehydrogenase (Idh*), lactate dehydrogenases (Ldh-1*, Ldh-2*), malate dehydrogenases (Mdh*), unspecific dehydrogenase (1 Locus), malic enzyme (Me*), mannose phosphate isomerase (Mpi*), 6-phosphogluconate dehydrogenase (6-Pgd*), phosphoglucomutases (Pgm-1*, Pgm-2*), superoxide dismutases (Sod-1*, Sod-2*), nucleoside phosphorylase (Pnp*). Following the structural analysis by Shinde and Furtado (1981) who found two α-globin chains in each electrophoretic fraction of sasin haemoglobin, and a different β-chain in each of two bands into which blackbuck haemoglobin is resolved, we count the haemoglobin pattern as representing the products of four loci.

Two plasma proteins, transferrin (Tf) and posttransferrin-2 (Ptf-2) showed three phenotypes each in the 49 investigated blackbuck: The banding patterns at both loci segregated in all cases of known family genealogy like biallelic genetic polymorphisms. The Tf-system comprised a second, faster-migrating variant Tf*107 of 107% relative mobility when referenced to the main allele Tf*100. Tf bands were identified by comparing patterns with reference alleles from several other ruminant species. All three possible genotypes were present (Tf*100/100, Tf*100/107, Tf*107/107), their numbers did not deviate from Hardy-Weinberg distribution (Fig. 1). The functional identity of Ptf-2 remains unknown, but the designation "posttransferrin-2" refers to its electrophoretic migration as the second major plasma protein following transferrin cathodically. In addition to the more common allele Ptf-2*100, the second variant Ptf-2*83 reached 83% mobility. The combinations Ptf-2*100/100, Ptf 2*83/100, and Ptf-2*100 were observed in numbers corresponding to Hardy-Weinberg expectations (Fig. 1). Glucose phosphate isomerase (Gpi-1*) variation displayed the typical three-banded heterozygous zymogramme of a dimeric enzyme with the alleles Gpi-1*100 and Gpi-1*300: The intermediate band of heterozygotes representing the heterodimere Gpi-1*100/Gpi-1*300 yielded double enzyme activity when compared with both homodimeres, the cathodal Gpi-1*100/Gpi-1*100 and the anodal Gpi-1*300/Gpi-1*300 (Fig. 1). This distribution of enzyme activites can be expected because the four possible combinations of two alleles (100/100, 100/300, 300/100, 300/300) are electrophoretically resolved into only three bands, both heterodimeres coalescing into a common band representing 50% of overall enzyme activity (against 25% for each homodimere). This typical zymogramme was not encountered for the variant Gpi-1*600 whose heterozygotes with Gpi-1*100 indicated a much lower staining intensity of the anodal homodimere (Gpi-1*600/Gpi-1*600) than for the cathodal Gpi-1*100/Gpi-1*100 (Fig. 1). The heterodimere Gpi-1*100/Gpi-1*600, however, indicates approximately two-fold enzyme activity when compared with the Gpi-1*600-homodimere. We conclude that despite an unexpected distribution of staining intensities between bands, Gpi-1*600 is a valid allele able to enter heterodimeric combinations with the common variant. It may be

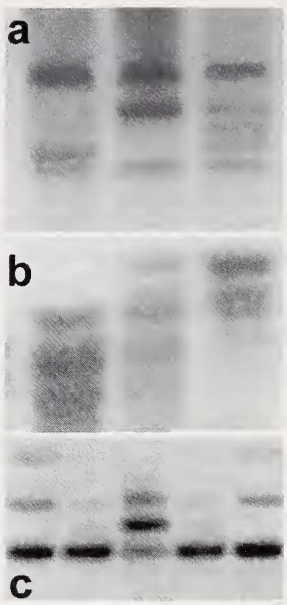

Fig. 1. Blackbuck (*Antilope cervicapra*). Three electrophorectic protein polymorphisms. 1 a. Posttransferrin-2 (Ptf-2*). Genotypes from left to right: Ptf-2* 100/100, Ptf-2* 83/83, Ptf-2* 83/100. 1 b. Transferrin (Tf*). Genotypes from left to right: Tf* 107/107, Tf* 107/100, Tf* 100/100. 1 c. Glucose phospate isomerase-1 (Gpi-1*). Genotypes from left to right: Gpi-1* 600/100, Gpi-1* 100/100, Gpi-1* 300/100, Gpi-1* 100/100, Gpi-1* 600/100. The negative pole is at the upper margin of micrographs 1 a and 1 b, and at the lower margin of micrograph 1 c.

either less strongly expressed and therefore be present in smaller quantities than the common allele, or this enzyme variant prefers biochemical conditions different to those supplied by our staining protocol, resulting in much weaker activities in our gels than would be expected for codominantly expressed alleles with a simple gene-dosage effect. As could be expected from allele frequencies, both Gpi-1*300 and Gpi-1*600 were confined to heterozygotes with Gpi-1*100; neither did we observe the genotype Gpi-1*300/600. Posttransferrin-1 produced variable patterns too, but the clear scoring of this system deteriorated with prolonged storage of frozen sera. Therefore, we neglect these variants until additional samples from pedigreed sasins are available to exclude non-genetic variability or storage artefacts.

Sample sizes, allele frequencies and heterozygosities of individual herds and of the combined metapopulation are listed in table 2. The fraction of polymorphic loci measured $P = 0.034$ for allozymes only, and $P = 0.075$ for enzymes and structural blood proteins. Heterozygosity was $H = 0.005$ for enzymes, and $H = 0.025$ for enzymes and serum proteins combined. These values are compared with published data on gazelle species listed in table 3.

Discussion

While Tf provides useful genetic marker alleles in several ruminants (Schreiber 1991), the homology and the function of post-transferrins remain unidentified. Gahne et al. (1977) described a very similar polymorphism from domestic taurine cattle, also operationally

Table 1. Buffer systems used to resolve allozymes of blackbuck. Gel buffers are mostly prepared by diluting the respective electrode buffers (EB).

Enzyme	Electrode buffer	Gel buffer
Lactate dehydrogenase (E.C. 1.1.1.27)	100 mM NaOH/0.3 M boric acid, pH 8.6	67 mM Tris/5 mM NaOH/5 mM citric acid/15 mM boric acid, pH 8.6
Malate dehydrogenase (E.C. 1.1.1.37)	250 mM NaH_2PO_4/150 mM citric acid, pH 5.9 with NaOH	EB 1:40, pH 5.9
Malic enzyme (E.C. 1.1.1.40)	100 mM Tris, pH 7.4 with NaH_2PO_4	EB 1:10, pH 7.4
Isocitrate dehydrogenase (E.C. 1.1.1.42)	250 mM NaH_2PO_4/150 mM citric acid, pH 5.9 with NaOH	EB 1:40, pH 5,9
6-Phosphogluconate dehydrogenase (E.C. 1.1.1.44)	690 mM Tris/160 mM citric acid, pH 8.0 with HCl	EB 1:30, pH 8.0
Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49)	110 mM Tris/60 mM boric acid/ 2.5 mM EDTA, pH 8.6 with NaOH	EB 2:5, pH 8.6
NADH diaphorase (E.C. 1.6.2.2)	110 mM Tris/60 mM boric acid/ 2.5 mM EDTA, pH 8.6 with NaOH	EB 2:5, pH 8.6
Superoxide dismutase (E.C. 1.15.1.1)	250 mM NaH_2PO_4/150 mM citric acid, pH 5.9 with NaOH	EB 1:40, pH 5.9
Purine nucleoside phosphorylase (E.C. 2.4.2.1)	100 mM NaOH/0.3 M boric acid, pH 8.6	67 mM Tris/5 mM NaOH/5 mM citric acid/15 mM boric acid, pH 8.6
Glutamate oxaloacetate transaminase (E.C. 2.6.1.1)	200 mM NaH_2PO_4/150 mM Borat, pH 7.0	EB 1:10, pH 7.4
Adenylate kinase (E.C. 2.7.4.3)	200 mM Tris, pH 8.0 with citric acid	EB 1:3, pH 8.0
Phosphoglucomutase (E.C. 2.7.5.1)	100 mM Tris/100 mM maleic acid/ 10 mM $MgCl_2$/10 mM EDTA, pH 7.4	EB 1:10, pH 7.4
Esterases (E.C. 3.1.1.1)	100 mM Tris/100 mM maleic acid anhydride, pH 7.2	EB 1:10, pH 7.2
Acid phosphatase (E.C. 3.1.3.2)	150 mM trinatrium citrate/240 mM NaH_2PO_4, pH 6.3	EB 1:40, pH 6.3
Adenosine deaminase (E.C. 3.5.4.4)	110 mM Tris/60 mM boric acid/ 2.5 mM EDTA, pH 8.6 with NaOH	EB 2:5, pH 8.6
Carbonic anhydrase (E.C. 4.2.1.1)	110 mM Tris/60 mM boric acid/ 2.5 mM EDTA, pH 8.6 with NaOH	EB 2:5, pH 8.6
Glyoxalase (E.C. 4.4.1.5)	200 mM Tris/HCl, pH 8.0	EB 1:10, pH 8.0
Mannose phosphate isomerase (E.C. 5.3.1.8)	100 mM Na_2HPO_4/100 mM NaH_2PO_4, pH 7.0	EB 1:10, pH 7.0
Glucose phosphate isomerase (E.C. 5.3.1.9)	250 mM Tris/60 mM citric acid, pH 7.5	60 mM Tris/2 mM citric acid, pH 7.5

called Ptf-2. There was no deviation from the expected family segregation in the known segments of the sasin pedigree, and both Ptf-2 variants certainly represent Mendelian alleles. Both polymorphic serum proteins and one triallelic isoenzyme may be useful markers for the genetic management of blackbuck populations. Gazelles are a group of ruminants whose management poses problems in both zoos and nature reserves by documented inbreeding depression (Templeton and Read 1984; Templeton et al. 1987) and controversial taxon definition (Granjon et al. 1991; Bigalke et al. 1993; Vassart 1994); different con-

Table 2. Protein polymorphism in captive-bred blackbuck. n = sample size; p_x, p_y, p_z = allele frequencies; H = observed heterozygosities (H_o) and their Hardy-Weinberg expectations (H_e).

		Tf			Ptf-2			Gpi-1*			
	n	p_{100}	p_{107}	H_o/H_e	p_{100}	p_{83}	H_o/H_e	p_{100}	p_{300}	p_{600}	H_o/H_e
total	49	0.774	0.226	0.340/0.354	0.565	0.435	0.500/0.496	0.918	0.072	0.010	0.163/0.152
Givskud	29	0.776	0.224	0.310/0.354	0.483	0.517	0.552/0.508	0.983	0.017	0.000	0.034/0.033
Cologne	11	0.773	0.227	0.455/0.368	0.636	0.364	0.545/0.485	0.864	0.136	0.000	0.273/0.267
Alborg	5	0.900	0.100	0.200/0.180	0.800	0.200	0.400/0.320	0.900	0.000	0.100	0.200/0.180
Rostock	3	0.500	0.500	1.000/0.500	1.000	0.000	–	0.500	0.500	0.000	1.000/0.500
Paris	1	1.000	0.000	–	0.000	1.000	–	1.000	0.000	0.000	–

Table 3. Variabillity of protein electromorphs in various gazelles. P = fraction of polymorphic loci. H = percentage of heterozygous patterns (for springbok and blackbuck, the expected heterozygosity is provided, calculated on the basis of allele frequencies, the other studies indicate values of observed heterozygosity). n. s. = not specified.

Species	Sample size	Loci	*P*	*H*	Authors
Thomson's gazelle					
Gazella thomsoni	33	40	0.196	0.055	Georgiadis et al. (1990)
Gazella thomsoni albonotata	8	16	0.187	0.085	Vassart et al. (1994)
dorcas gazelle					
Gazella dorcas	25	16	0.187	0.074	Vassart et al. (1994)
Springbok					
Antidorcas marsupialis	24	46	0.174	0.060	Bigalke et al. (1993)
Speke's gazelle	27	19[1]	0.158[1]	n. s.	Templeton et al. (1987)
Gazella spekei		28[2]	0.143[2]		
goitred gazelle					
G. subgutturosa marica	30	16	0.125	0.021	Vassart et al. (1994)
	30	20	0.150	0.017	Granjon et al. (1991)
mountain gazelle					
G. g. gazella	16	16	0.062	0.027	Vassart et al. (1994)
	14	24	0.167	0.050	Vassart et al. (1995 a)
G. g. cora	7	16	0.062	0.008	Vassart et al. (1994)
	2	24	0.208	0.088	Vassart et al. (1995 a)
G. g. farasani	5	16	0.062	0.030	Vassart et al. (1994)
G. g. erlangeri	15	16	0.000	0.000	Vassart et al. (1994)
	2	24	0.000	0.000	Vassart et al. (1995 a)
blackbuck	49	29[1]	0.034[1]	0.005[1]	this study
Antilope cervicapra	49	40[2]	0.075[2]	0.025[2]	

[1] = isoenzyme loci
[2] = isoenzymes, haemoglobins and plasma proteins

cepts are available on sasin microtaxomomy (Zukowsky 1927, 1928; Groves 1982). An inhabitant of open grassy plains, sasin initially profited from human agriculture clearing dry forests, and historic concentrations of 10 000 specimens, or more, have been observed on single cattle ranches (Jerdon 1874). Total population estimates from the past are unreliable but an Indian nobleman alone kept 1 000 tame cheetahs chiefly for blackbuck hunting (Ranjitsinh 1989). Centennial reductions left only 43 500 blackbuck for India in 1989, scattered over numerous, isolated and generally rather small herds (Ranjitsinh 1989). Feral stocks in Texas and Argentina number a couple of thousands, they are used, together with zoo-bred sasin, to repopulate extirpated herds in Pakistan (Ranjitsinh 1989). This conservation management would profit from more extensive knowledge of population variability and differentiation to select proper herds for restocking.

Compared with other Antilopinae, blackbuck proteins proved fairly homozygous. Even when Tf and Ptf-2 are considered, loci which have been studied only rarely by previous investigators of gazelline polymorphism, sasins range at the lower end of previously observed allelic diversities. Small samples from captive mountain gazelles (*G. gazella*) showed similar or lower variability at 16 loci but Vassart et al. (1995 a) raised the documented variability of two subspecies of *G. gazella* when increasing the number of sampled loci from 16 to 24. Small specimen and locus samples easily distort allele frequencies, and differences in electrophoretic methods might influence the comparison of allozyme data too. Still, the available evidence proposes that blackbuck contains less genetic variation as a species than do most true gazelles. As an alternative, continued captive breeding has eroded the genetic variation from a previously more polymorphic state. We cannot decide this alternative with certainty without including samples from wild populations, but relevant arguments are summarized.

Kept in large groups, many blackbuck are unknown to zoo curators as individuals, and studbook management is absent. Captive-bred for the first time in 1888 (Schwarz 1980), the origins of modern herds predates the time when detailed records were documented in zoological gardens. Therefore, we are ignorant of many details of the breeding history. There are exceptions like Tierpark Berlin which has bred 250 blackbuck from up to nine male and 12 female founders by 1995 (Pohle 1995), and the Rostock Zoo herd whose history is also fairly well documented (Schwarz 1980; K. Linke, pers. comm.). Hamadryas baboon (*Papio hamadryas*), another traditional zoo species in which few dominant males reproduce in any colony, revealed allozyme polymorphism and heterozygosity reduced by possibly 75% and 80% respectively, after 30 years of captive breeding without a studbook (Wang and Schreiber 1996). Different to baboons (Wang and Schreiber 1996), allele frequencies which would indicate genetic drift between zoo stocks were not observed in blackbuck. The stocks at the zoos of Cologne, Givskud, and Rostock had seen the import of unrelated individuals prior to sampling. Founder effects when establishing the zoo population decades ago might also have been moderate because representatives of both blackbuck subspecies (Groves 1982) may be discerned among current zoo phenotypes. *A. c. rajputanae* from northwest India and formerly Pakistan have larger skulls, the sable-coated males have a grey sheen, rough long hair, and broad white rings surround the eyes; *A. c. cervicapra* from central-south India has shorter skulls, a narrow eye-ring, and shorter fine hair (Groves 1982). Based on some 100 blackbuck imported into European zoos in the 1920 s, Zukowsky (1927, 1928) described four different species. This splitting attitude has been reconciled with modern taxonomy by Groves (1982) who lumped these species into two subspecies, but from the geographic information and the accompanying photographs by Zukowsky (1927, 1928) one may conclude that sasins from all over India have entered the European zoo population. The smaller type with weakly divergent, open-spiralled shortish horns prevails in contemporary zoo herds, but blackbuck matching the phenotypes of northern populations are also included in our sample which includes descendants from at least twelve zoo herds. Since zoos chiefly exchanged breeding males, many more captive-bred sasin are cross-bred between lineages than is superficially indicated by the numbers of import of unrelated individuals. Moreover, the other published studies of electrophoretic variation in gazelles listed in table 3 also refer to captive specimens with a single exception (Bigalke et al. 1993), and sometimes to inbred stocks indeed (Templeton and Read 1984; Templeton et al. 1987).

Ranjitsinh (1982, 1989) described lekking in Velavadar National Park (NW India), where 1 300–1 700 sasin occur at a density of one per hectare (Rashid 1978; Jhala et al. 1992): 48–52 adult male sasins crowded on a display ground of 680×430 square metres, with a core area of 385×290 square metres comprising 30 territories. Microterritories in the sasin arena are advertised by postures and motions of the black-and-white bucks, and by pellet heaps in their centres on which bucks may sit down (Ranjitsinh 1989). Lekking

implies the occupation of periodically established display territories where males aggregate solely for course of the rutting season, the most dominant buck typically conquering privileged sites which are preferred by mate-seeking females. Lekking behaviour is sufficiently common and conspicuous in sasin for Gujarati people to use a particular designation for small lek territories, *akhlis* (Ranjitsinh 1982). Lekking was also observed in Chapar Tal Sanctuary, Ratjastan (Ranjitsinh 1989) and at Guleria, Nepal (Bauer and Ellenberg 1993) but appears to be inconspicuous elsewhere (Schaller 1967; Meier et al. 1973; Lehmkuhl 1981; Bharucha and Asher 1993; Bauer and Ellenberg 1993). Lehmkuhl (1981) and Bauer and Ellenberg (1993) suggested that reproductive behaviour of sasin was density-correlated, that lekking may be confined to aggregations in good habitats, and that male monopolized stable female breeding groups when population density was low. Observers of zoo-living or feral blackbuck restrained in enclosures expectedly did not encounter leks, but agree on polygynous sociology and mating competition (Meier et al. 1973; Dubost and Feer 1981; Mungall 1991). Ranjitsinh (1989) observed the longest horns and darkest pelage in dominant males with successful access to receptive females. The display of black coats in open sun-dry semideserts (at temperatures rising to 48 °C in the shade) must confer physiological stress by heat absorption and water deprivation (Jhala et al. 1992), a disadvantage which appears to be outweighted by sexual selection (Ranjitsinh 1989). Polygynous promiscuity is detrimental to the preservation of population polymorphism because many males are excluded from siring offspring.

Fisher (1930) and Lande (1981) developed a model of "run-away-evolution" for species with strong sexual selection by females on male polygenic traits. Such species would evolve sexual dimorphism in signalling characters. Distorted contribution to reproduction by few males fulfilling female selection criteria would result in small genetically effective population sizes narrowing variability. Ultimately, frequent speciation events result from small changes in criteria of female choice in certain populations. There are only few prerequisites to fulfil this model of "runaway evolution", e. g. genetic linkage of inborn female selection criteria for particular male phenotypes with the loci determining those male appearances. The length and form of sasin horns appear to vary on a local rather than on a macrogeographic scale, but there is a tendency for the longest, most divergent, and most closely spiralled horns to occur in males of the subspecies *A. c. rajputanae* which inhabits the region where lekking populations have been identified (Groves 1982; Ranjitsinh 1989). Twenty-one of the 24 top hunting trophies harvested from sasin originated from the area occupied by this population (Ranjitsinh 1989). The model by Lande (1981) predicts a speciose clade of genetically rather monomorphic taxa. Fossil spiral-horned gazelles resembling blackbuck in horn form, dentition or crania are known from Pliocene and Pleistocene deposits, i. e. *Helicotragus*, *Palaeoreas*, *Protragelaphus*, *Antilospira*, *Gazellospira*, *Spirocerus*, *Prostepsiceros*, and *Antilope subtorta* (Pilgrim and Schaub 1939; Thenius 1969; Gentry 1970, 1971, 1976). This diversity of fossil spiral-horned genera which may or may not reside in sasin's ancestry could corroborate the rapid typogenesis predicted by the Lande (1981) model. As a contrast to the vivid speciation in spiral-horned gazelles, Miocene fossils from Europe and North Africa testify to the greater age and temporal continuity of *Gazella*, the inferred paraphyletic genus from which blackbuck appears to have descended (Effron et al. 1976; Vassart 1994; Vassart et al. 1995 b).

Correlations between biochemical polymorphism and lifestyle cannot prove causal connections. Field work on sasin sociology and large-scale population genetic inventories of free-living local populations, both lekking and non-lekking, are needed to confirm that the models of Fisher (1930) and Lande (1981) provide an explanation for the microevolution of blackbuck. However, in a period when decisions are required of how to manage the genetic variability of disappearing wildlife, the possible correlation between mating system (or other life-history traits) and allelic variability calls for cautious interpretation when finding high or low levels of allelic variability in studbook populations of relict

stocks (Sugg et al. 1996). Conservation breeding plans would profit from a better knowledge of genetically effective population sizes to estimate the level of inbreeding which is typical of free-living blackbuck populations.

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Zusammenfassung

Niedrige Allozym-Heterozygotie in Hirschziegenantilopen aus Zoologischen Gärten

Die elektrophoretische Analyse von 40 Blutproteinen bei 49 zoolebenden Hirschziegenantilopen (*Antilope cervicapra*) erbrachte die für Gazellen niedrigen Werte von P = 0.075 (P = 0.025 nur für Enzymloci) für den Anteil polymorpher Loci und H = 0.034 (H = 0.005 für Allozyme) für die beobachtete Heterozygotie. In Ermangelung von Vergleichsproben aus Freilandbeständen kann ein varianzmindernder Einfluß der Zucht in Gefangenschaft nicht ausgeschlossen werden. Jedoch sprechen paläontologische Befunde (rasche Typenbildung) ebenso wie der für eine Gazelle außergewöhnliche Sexualdimorphismus und das bei Antilopinae einzigartige Sozialsystem (Arenabalz) dafür, daß die Hirschziegenantilope einer raschen Mikroevolution unterliegen sollte. Geringe genetische Effektivpopulationen, verbunden mit sexueller Auslese, wären Faktoren der arteigenen Lebensweise, welche die genetische Variabilität absenken.

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Authors' addresses: Dr. Arnd Schreiber, Dipl.-Biol. Peter Fakler, Zoologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 230, D-69120 Heidelberg; Dr. Richard Østerballe, Løveparken Givskud, Give, Denmark